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Primary Research Paper

Incidence of mentum deformities in midge larvae (Diptera:Chironomidae) from Northern Nova Scotia, Canada

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Abstract

Deformities in the mouthparts of larval Chironomidae, particularly of the teeth on the mentum, have been proposed as a bioindicator of sediment quality and environmental stress. Most work to date has concentrated on relatively few abundant, responsive genera common in soft-bottom lakes. We examined mentum deformities in 25 genera of Chironominae, Orthocladiinae and Diamesinae (one genus) from streams and a lake in rural Nova Scotia where farming and forestry are the principal land uses. Incidence of deformity at similar stream sites varied across genera from zero to >10%. Average frequencies of deformity across all three subfamilies at sites with no known sources of contamination ranged from <4% to 8%, and increased to nearly 15% at a site receiving treated municipal sewage effluent. Differences in chironomid community structure and rates of leaf litter decomposition above and below the sewage effluent outfall were congruent with the difference in mentum deformities. Frequencies of deformity observed here are an order of magnitude greater than in similar studies of rural areas. Low-level stress from agriculture or forest harvesting may be widespread in rural regions even in aquatic ecosystems that are seemingly free of industrial discharges or sediment contamination.

Introduction

Late-instar larvae of Chironomidae (non-biting midges, order Diptera) frequently develop deformities in the mouthparts, especially the mentum, when exposed to stress or pollution. The frequency of deformity can be used to assess sublethal sediment toxicity associated with heavy metals, radioactivity, oganophosphate or organochloride pesticides, and other xenobiotics (Bird et al., 1995; Servia et al., 1998; Swansburg et al., 2002; Watanabe et al., 2000; Mori et al., 1999; Vermeulen et al., 1995; Martinez et al., 2002). With respect to metals, mentum deformities have been used to indicate the presence of copper, lead, zinc, cadmium, nickel, arsenic, and mercury in water and sediment (Janssens de Bisthoven, 1998a; Servia et al., 1998; Mori et al., 1999; Mergalli et al., 2000; Vermeulen et al., 2000; Watanabe et al., 2000; Martinez et al., 2001, 2002, 2004; Swansburg et al., 2002). The presence of developmental deformities appears to be a general stress response to a wide range of environmental contaminants (Vermeulen, 1995).

The Chironomidae is a large family of an estimated 2000 species in North America (Coffman & Ferrington 1996). Nevertheless, most research concerning mouthpart deformities in chironomids has concentrated on midges from the genus *Chironomus*. Within this genus, *Chironomus riparius* and *Chironomus tentans*, two large, widespread species characteristic of soft-bottomed sediments, are frequently studied (e.g., Bird et al., 1995; Janssens de Bisthoven, 1998a, b; Martinez et al., 2001; Vermeulen et al., 2000; Martinez et al., 2004). Relatively little is known about frequency of mouthpart deformities in other genera within this exceedingly diverse family. Excepting three studies discussed below (Bird, 1994; Canfield et al., 1996; Swansburg et al., 2002), deformities have been reported in fewer than two dozen species among the many hundreds within the family Chironomidae.

Several limitations curtail routine use of chironomids as biomonitoring agents. First, fine details of mouthparts may be difficult to see in early instar larvae or smaller species without sophisticated microscopy. The taxonomic challenges of this speciose group may limit the applicability of the method to those who have access to expertise. Moreover, midge species vary in their tolerance to contaminants (Hamilton & Sæther, 1971; Hare & Carter, 1976; Wiederholm, 1984) and in their propensity to develop deformities (Servia et al., 1998; Janssens de Bisthoven, 1998b; Vermeulen et al., 2000; Martinez et al., 2001; Martinez, 2004). To determine if a location under inspection is polluted, the background incidence of deformity must be accounted for, because some larvae are deformed naturally. Our lack of knowledge of the frequency of deformities in natural, unstressed environments, especially for species other than those commonly studied, hinders application of this biomonitoring method (van Urk et al., 1992).

At the same time, sediment contamination or other pollution may eliminate certain species entirely. While disappearance of a species may also be used to indicate pollution, it complicates the quantitative application of mouthpart deformities if different species, with different background deformity frequencies, are found in clean and polluted areas. If the chironomid community is diverse, however, it may still be possible to determine a difference in mean deformity frequency by averaging results from several taxa.

A few studies on chironomid deformities have taken this tack (Bird, 1994; Canfield et al., 1996; Swansburg et al., 2002). All of these studies examined chironomids in rivers, where a more diverse community is usually present than in standing waters. Bird (1994) examined some 30 species of chironomid and Swansburg et al. (2002) looked at 50. These researchers were able to use chironomid community structure, as well as deformity frequencies, to evaluate environmental contamination. A more confident conclusion can be drawn when these two measurements are congruent.

In this study, an investigation of mouthpart deformities in Chironomidae from a variety of aquatic habitats in northern Nova Scotia, Canada, was undertaken. The main objectives were to determine if there is a common background incidence of deformity among genera, and to see if a community-level difference in deformity frequency could be detected among chironomids exposed to a mild stress, treated domestic sewage effluent. The deformities examined were misshapes in the median or lateral teeth of the mentum; these were the most common deformities and the easiest to see. Simultaneous data on deformities and community structure could help bridge the association between deformities and pollution.

Background incidence of deformity was here defined as that occurring in water bodies apparently free from point-source inflows (e.g., wastewater effluents) or historical contamination. All these rivers and lakes lie in a rural, forested landscape disturbed only by short-rotation forest harvesting and mixed farming; three sites were within the limits of a small town. Consequently, the background incidence of deformity was anticipated to be low, on the order of 1% (Wiederholm, 1984; Warwick et al., 1987; Bird, 1994; Swansburg et al., 2002). We hypothesized that treated sewage effluent might produce an elevation of deformity frequency if it contained residual metals or other trace contaminants not removed by treatment.

A secondary objective of this work was to test two extensions of the current method for assessing chironomid deformities. In the field, we used decomposing leaf litter as an artificial substrate to encourage colonization by chironomids. Mass loss from the leaf litter provided a third, independent indicator of biological effects of contaminants. In the laboratory, we examined chironomid mouthparts under a scanning electron microscope (Wiederholm, 1984), as well as with the dissecting and compound microscopes traditionally used. The electron microscope provided a detailed view of the teeth which could not be seen using the ordinary light microscope. It also permitted inspection of small species and early instars.

Materials and Methods

Chironomid larvae were collected from various aquatic habitats in and around Antigonish, Nova Scotia, from May to August, 2004. These collections were supplemented by specimens collected in other studies in 2002 and 2003. Chironomid sampling was of two types: (1) background sampling of streams and lakes to establish frequencies and degrees of deformity and (2) experimental study of effects of treated sewage effluent. Background samples were collected from a drainage ditch (Mall Pond inlet), three local streams (Brierly Brook, Ogdens Brook, Wrights River), a swamp-stream (Pictou Landing) and a productive lake (Gaspereaux Lake).

The Mall Pond inlet was a small drainage ditch carrying ground water and roadside runoff down a steep slope into a pond. The sampling site was a fast-moving riffle with a rocky bottom. Chironomids of the genus *Diamesa* were collected by hand-picking them off a rock selected from the middle of the riffle.

Brierly Brook, a third-order, cobble-bottomed stream draining mixed forest and farmland, is typical of many such water courses in rural Nova Scotia. There are no industrial inflows to the stream, but it has suffered extensive channel modification historically. Forestry and mixed farming are the major land uses in the basin. The lower reach of the brook flows through the Town of Antigonish (population 5000). Brierly Brook was sampled in 2004 at several locations using a D-net or a Surber sampler, or by picking individual animals off rocks with fine forceps. Further samples were collected at three locations along Brierly Brook with a Surber sampler in June 2003 as part of another study.

Ogdens Brook is similar to Brierly Brook except that it flows through a steep, forested valley for much of its length. A total of 49 chironomids were collected from decomposing leaves taken from various riffles along the brook.

The swamp-stream at Pictou Landing is described in detail in Taylor & Dykstra (2005). A shallow, slow-flowing rivulet drains a corridor of wet forest and swamp dominated by speckled alder (*Alnus incana*) and red maple (*Acer rubrum*). Chironomids were collected from artificial substrates (stone-filled trays) placed in the stream or from samples of decomposing alder leaf litter in June and July, 2002.

One hundred and ten chironomid larvae were gathered from Gaspereaux Lake. The 1-km^2 lake is shallow (maximum depth < 5 m) and very productive, with a soft bottom of sand and muck. Approximately a dozen houses and cottages are situated near one shore; the rest of the basin is forest and fields. The lake was sampled at several locations with an Ekman grab. All the chironomids taken from Gaspereaux Lake were of the genus *Chironomus*.

Wrights River, a wide, cobble-bottomed stream comparable in setting and hydrology with Ogdens and Brierly brooks, receives treated sewage effluent from the Town of Antigonish. This stream is largely unshaded in its lower reaches, and meanders through a series of riffles and runs toward Antigonish Estuary. Samples were collected above and below the sewage effluent outfall with D-nets and a Surber sampler, or by picking individual chironomids off rocks with forceps. Chironomids were also collected at this site by allowing them to colonize decomposing leaf litter, as described next. A total of 588 chironomids were taken from Wrights River.

The second part of the sampling program quantitatively compared frequencies of chironomid deformity above and below the effluent outfall from the Town of Antigonish. Effluent from the Antigonish domestic sewage system (aerated lagoon) is released at a rate of approximately 100 l/min after sterilization with ultraviolet light. The effluent is clear, circumneutral and typically high in dissolved oxygen (>10 mg/L). In laboratory experiments, undiluted effluent had a 5-day biochemical oxygen demand of 4.8 ± 0.3 mg/L (SD) and strongly stimulated growth of duckweed (*Lemna minor*).

The Antigonish effluent enters Wrights River near the mouth, at a transition between typical riffle-run habitat and a deep pool (>1.2 m) at the edge of the estuary. Chironomids were collected from shallow water immediately above (Upstream site) and below (Effluent site) the outfall, where effluent effects would be strongest. A few upstream specimens were collected in similar habitat about 0.5 km upstream from the sewage treatment plant. A third site (Downstream) comprised the deep pool < 10 m below the effluent outfall, from which chironomids were collected with a long-handled D-net.

Benthic populations of chironomids below the sewage effluent outfall were conspicuously low, especially in June. Consequently, in addition to benthic sampling, leaf packs were used as artificial substrates to compare chironomid colonization at the Upstream and Effluent sites. Leaf packs were contained within numbered, fibreglass mesh bags with a mesh size of 2 mm (Taylor and Dykstra, 2005). Each of the 10 bags contained 2.0 ± 0.05 g of air-dried, autumn-fallen leaves of red maple, a common stream-side species. On 22 July 2004, 5 bags were placed on the stream bottom about 10 m above the effluent outfall (Upstream site) and pinned in place with galvanized nails. The other 5 bags were placed in the mixing zone below the outfall, along a gravel bar formed by the effluent outflow (Effluent site). After 3 weeks, 4 bags from each site were removed from the stream, placed in sealable plastic bags with some stream water and returned to the laboratory in a cooler. Two bags could not be located.

In the laboratory, chironomids (and other invertebrates) were removed by thoroughly rinsing the leaves with tap water onto a 500 μm sieve. Individual animals were removed with fine forceps. Litter samples were then transferred to preweighed paper bags, dried in an oven at 70 °C to constant weight, cooled in a desiccator and reweighed to determine mass remaining. Ten air-dried samples of fresh litter were oven-dried to calculate a correction factor (0.940) to convert original air-dried mass to oven-dried mass.

Chironomid larvae were preserved in 70% ethanol. Larvae were identified, usually to genus, using Coffman & Ferrington (1996) and Peckarsky et al. (1990), except Tanypodinae, which were identified to subfamily. The genus *Chironomus* was subdivided into two morphologically distinguishable but unnamed species.

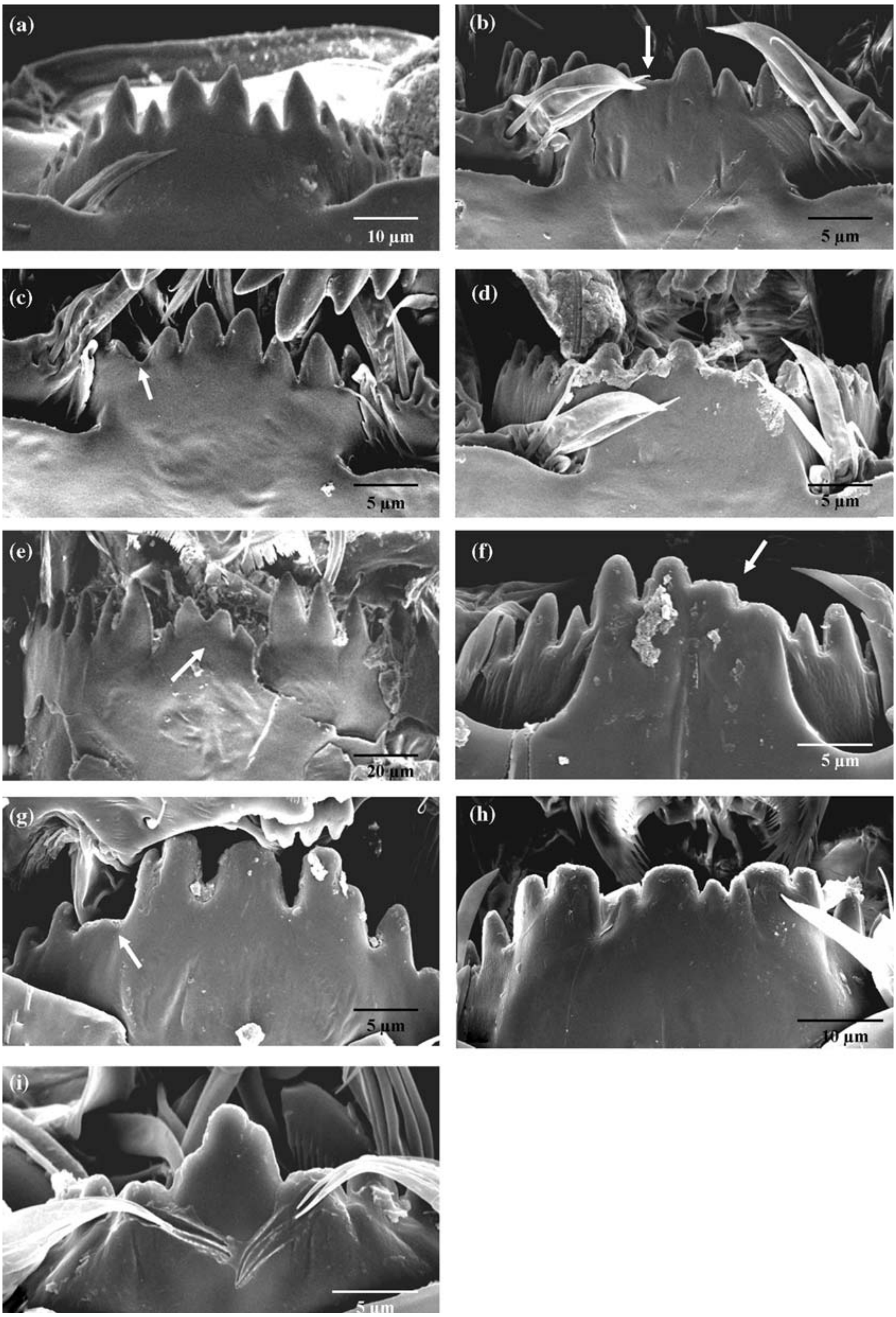
Larger animals were examined under a dissecting microscope. Mouthparts of some of the smaller genera were examined under a compound light microscope at 100× or 400× magnification. Any chironomids that had deformities in their teeth were examined in greater detail (1000× to 2000× magnification) under a Jeol JSM 5300 scanning electron microscope operated at 15 Kv. The mentum was examined for missing or clearly mis-shaped teeth or asymmetries between sides. We classified each specimen as deformed or normal, without finer qualification. Teeth that showed signs of breakage (sharp edges) were not included as deformities.

Results

A total of 947 chironomid larvae were examined in the course of this work. In addition to members of the sub-family Tanypodinae, in which genera were not distinguished, a total of 25 identifiable genera of chironomids were captured, 13 in the Chironominae, 11 in the Orthocladiinae and one (*Diamesa*) in the Diamesinae. The distribution of genera was scattered; most genera occurred at only 1–3 sites, and none occurred at all 8 sites. Seven genera, *Cryptochironomus*, *Eukiefferiella* , *Georthocladius*, *Heterotrissocladius*, *Paralauterbournellia*, *Paratendipes* and *Sublettea*, are represented by 5 or fewer specimens, all without deformities, and are not discussed further, although they are included in site totals.

The mentum in the subfamily Tanypodinae is reduced and obscure. Deformities have been reported in the ligulae of Tanypodinae (Warwick, 1991; Diggins & Stewart, 1993; Bird, 1994), but we found none in 119 specimens. Therefore, we considered only members of the Orthocladiinae,

Figure 1. Scanning electron micrographs of mentum deformities observed in chironomid larvae from northern Nova Scotia, Canada. (a) Normal mentum of *Polypedilum* from Ogdens Brook, June 2004 (×1000). (b) Mentum of *Polypedilum* missing median tooth (arrow), from upstream Wrights River, July 2004 (×1500) (c) Mentum of *Polypedilum* with mis-shaped lateral tooth (arrow), from effluent mixing zone in Wrights River, July 2004 (×1500). (d) Severely deformed mentum of *Polypedilum*, from effluent mixing zone in Wrights River, July 2004 (×1500). (e) Mentum of *Chironomus* showing poorly developed median teeth (arrow), collected from Gaspereaux Lake, July 2004. (×500) (f) Mentum of *Endochironomus* missing part of right median teeth (arrow), from Wrights River downstream from the effluent outfall, July 2004 (×1500). (g) Mentum of *Dicrotendipes* missing lateral tooth (arrow), from effluent mixing zone in Wrights River, July 2004 (×1000). (h) Severely deformed mentum from subfamily Chironominae, from Wrights River downstream from the effluent outfall, July 2004 (×1000). (i) Severely deformed mentum from subfamily Chironominae, from upstream Wrights River, July 2004 (×2000).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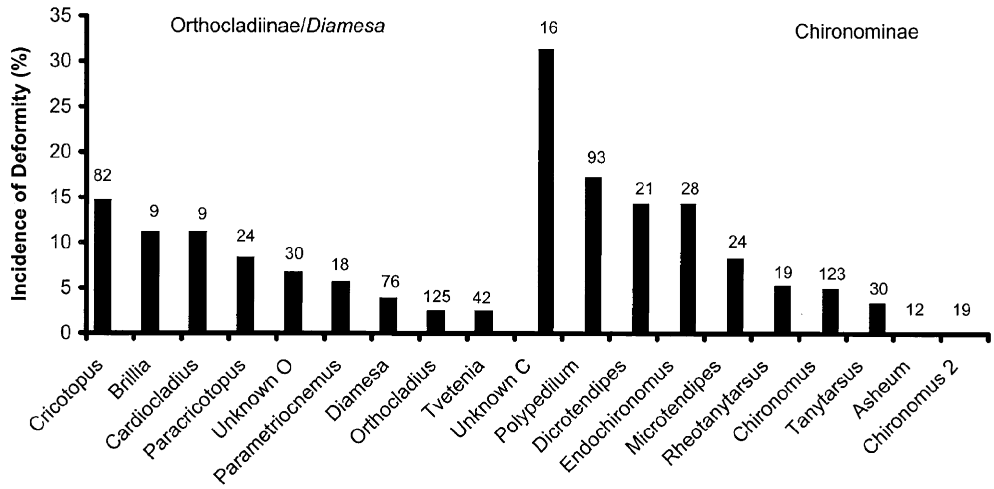

Figure 2. Incidence of mentum deformities in various genera of larval chironomids from northern Nova Scotia. Only genera represented by > 5 individuals or at least 1 deformed specimen are shown. *Diamesa* (subfamily Diamesinae) is shown among the Orthocladiinae. Unknown O and Unknown C refer to specimens within the Orthocladiinae or Chironominae which could not be identified further because of small size or severe deformity. Numbers above each column are sample sizes.

Chironominae and Diamesinae when calculating mean incidence of deformity across species for individual sites.

Although there was substantial variation among genera, deformities in the mentum teeth of chironomidae occurred relatively frequently. Of 763 specimens of all species from all sites (excluding Tanypodinae), 64 (8.4%) had observable deformities. Deformities ranged in severity from single teeth mis-shaped (Fig. 1c) or missing (Fig. 1b, f, g) to loss or malformation of all the teeth of the mentum (Fig. 1d, e, h, i). In a small number of specimens, the deformity was so severe that confident identifications to genus could not be made (Fig. 1h, i). The same kinds of deformities were observed in all the genera examined, illustrating that it is a general developmental anomaly. For example, in Fig. 1 the same deformity of the median tooth is illustrated in *Chironomus* collected from Gaspereaux Lake (Fig. 1e), and in *Polypedilum* collected from Wrights River (Fig. 1c).

Incidence of deformity varied widely among genera (Fig. 2). Among the subfamily

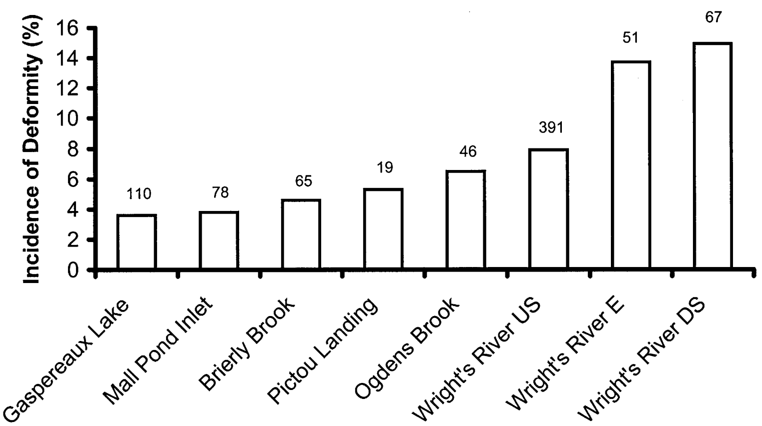

Figure 3. Incidence of mentum deformities in larval chironomids in all genera, excluding Tanypodinae, at lake and river sites. Three sites on Wrights River are shown, upstream (US), near the effluent outfall (E) and downstream (DS) from the Antigonish sewage treatment facility. Numbers above each column are sample sizes.

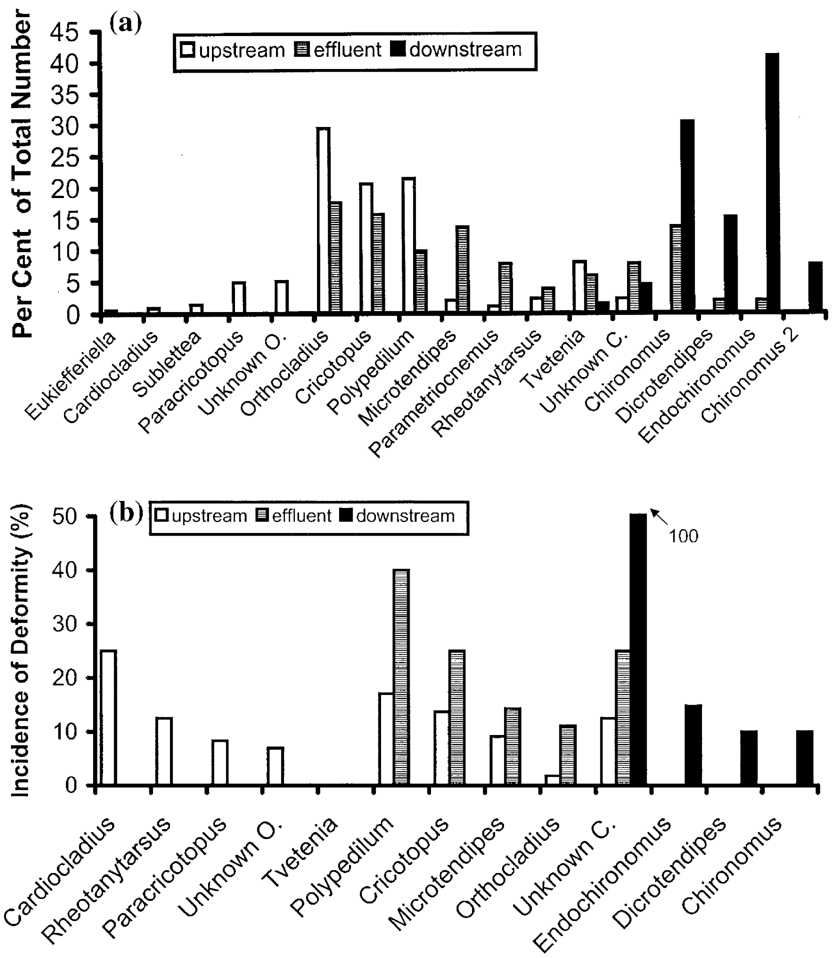

Figure 4. Effect of treated domestic sewage on chironomids in Wrights River, Antigonish. (a) Community composition from benthic and leaf-pack samples collected Upstream, near the Effluent outfall, and Downstream from the Antigonish sewage treatment facility. Values are proportions of each taxon, to allow for unequal sampling effort at different sites. Unknown O and Unknown C refer to specimens within the Orthocladiinae or Chironominae which could not be identified further because of small size or severe deformity. (b) Incidence of deformity in common chironomid genera. Only genera represented by >5 individuals and at least 1 deformed specimen are shown.

Chironominae, the most prominent taxon is the "Unknown" group (Fig. 2). Along with specimens that were small or cryptic (11 animals), this taxon includes 5 specimens that were too deformed to be classified further. Among the identifiable genera, the incidence of deformity ranged from almost 15% in *Polypedilum*, *Dicrotendipes* and *Endochironomus* to zero in *Asheum* and *Chironomus* 2, which were each represented by fewer than 20 specimens. The more common species in the widely used genus *Chironomus* had a background incidence of deformity of only 5%. The range was similar in the subfamilies Orthocladiinae and Diamesinae (Fig. 2), except that no taxon was found without deformities, and there were far fewer unidentifiable animals. Several of these estimates of deformity incidence must be considered provisional because of small sample sizes. Nevertheless, 7 of the 19 taxa in Fig. 2 demonstrate natural deformity frequencies in excess of 10%, even where sample sizes are respectable (20 to >90 animals).

Overall incidences of deformity, calculated as the percentage of deformed menta among specimens of all genera, varied over a relatively small range among sites (Fig. 3). Deformity frequencies among chironomids from benthic collections and leaf packs in Wrights River were similar, so the data have been combined. The lowest frequencies were observed in Gaspereaux Lake (all *Chironomus*) and in the Mall Pond inlet (all *Diamesa*), where the incidence of deformities was $< 4\%$. The frequency of deformity in Brierly Brook, Ogdens Brook and Pictou Landing, roughly 5–7%, are probably typical for rural streams and rivers in this region.

A noticeably higher incidence of deformities is apparent in Wrights River, especially at the two sites below the effluent outfall, which approached 14–15% (Fig. 3). The upstream site on Wrights River, expected to be similar to Brierly or Ogdens brooks, shows a slightly higher incidence of deformities (7.9%), based on almost 400 specimens. While different species above and below the outfall may show different frequencies of deformity in response to stress, it seems reasonable to suppose that the average sensitivity across several species would be approximately the same. Therefore, if the frequencies of deformities calculated for the other sites can be taken as replicate measurements of the regional frequency at uncontaminated sites, the incidence of deformity in Wrights River can be compared using a simple *t*-test.

By this means, the incidence of deformities at the two sites below the effluent outfall in Wrights River is significantly higher than at the other sites ($t = 7.1$, $P < 0.01$, $n = 8$). However, the incidence at the upstream Wrights River site, which was anticipated to be unstressed, is also significantly greater than the mean for the sites on other water bodies ($t = 9.0$, $P < 0.01$, $n = 6$). Hence, a preliminary analysis suggests a source of stress affecting all of lower Wrights River, (which bounds Antigonish), but which is decidedly more severe below the sewage effluent outfall.

Closer evaluation of the effects of the Antigonish sewage effluent on Wrights River is complicated by the occurrence of different chironomid species downstream than upstream. A total of 13 taxa were collected above the outfall, and 11 in the mixing zone (Effluent site), but only 7 taxa occurred at both locations (Fig. 4A), as *Paracricotopus*, unknown Orthocladiinae, and three minor genera upstream were replaced by *Endochironomus*, *Dicrotendipes* and *Chironomus* near the outfall. The similarity of the chironomid communities at these sites may be compared using the Percent Similarity Index (Hruby, 1987), which incorporates both species composition and relative numbers. By this index, the Upstream and Effluent sites are only moderately similar (index = 0.57). The downstream site supported some of the species from the Effluent site, along with a second species of *Chironomus*. This site was very dissimilar from either the Effluent (0.24) or the Upstream sites (0.04). At least some of these differences in community structure are presumably effects of the effluent.

The distinction among the three sites is more apparent when the data are expressed as incidence of deformities among the common species (Fig. 4b). No statistical comparison among sites is possible using taxa that occur at only one site. However, in each of the 5 taxa found at both the Upstream and Effluent sites, the incidence of deformity in the mixing zone is always greater than that upstream (Fig. 4b). A paired *t*-test based on these 5 species indicates a significant difference in incidence of deformity ($t = 2.3$, $P < 0.05$). A chi-square test based on combined data leads to an identical result ($\chi^2 = 4.16$, $P < 0.05$). The evidence thus suggests a mild stress below the effluent outfall, which is manifested in a change in species composition and an increase in the incidence of mentum deformities.

Red maple leaf litter placed in the mixing zone of the sewage effluent outfall shows a much lower decomposition rate then litter placed above the outfall. Mass loss averaged $52.6 \pm 5.8\%$ above the effluent outfall, but only $27.6 \pm 7.9\%$ below, a highly significant difference ($t = 5.1$, $P < 0.01$, $n = 6$). Given that red maple can lose 25% of its mass within a few days of immersion through simple leaching (Taylor & Bärlocher 1996), these results suggest that leaves below the effluent had barely begun microbial decomposition in three weeks. Fortuitously, the missing downstream litter bag was recovered, intact, on 8 October 2004, by which time it had lost 55% of its original mass. Although unreplicated, this sample suggests it took 11 weeks for leaf litter in the effluent plume to reach the same state of decomposition reached in 3 weeks upstream.

Discussion

It is clear from this work that deformation of the mentum is a widespread developmental anomaly in Chironomidae, at least in the subfamilies we examined. Therefore, assessments of sediment contamination or other stresses using mouthpart deformities could easily be extended to virtually any water body, standing or flowing, which supports a population of chironomids. Most of the midge species which have seen routine use to date are characteristic of soft-bottomed lakes and large rivers (Coffman & Ferrington 1996). In many productive lakes, *Chironomus* may be virtually the only chironomid over most of the lake bottom, but its population density can be high, facilitating sampling. In riverine environments, where diversity is greater and population densities lower, a different approach may be called for.

The scanning electron microscope proved useful in this study for confirming and illustrating deformities, especially on the smallest specimens. The benefits of this instrument are the greater magnification and much finer resolution compared with conventional light microscopes; also, photomicrographs of chironomid mouthparts (Fig. 1) can provide a permanent record of both deformed and normal menta. Specimens are relatively quick to prepare because hard body parts require no fixation.

Nevertheless, some additional labour is involved when specimens are to be viewed under the scanning electron microscope: the head must be excised, mounted on a metal stub, air-dried, and sputter-coated with gold. These steps become time-consuming when many animals are examined. Ordinary light microscopy (whole animals mounted on slides) was always sufficient in our study to identify specimens and detect deformities, even for the smaller species. A dissecting microscope at 50× magnification was sufficient for a few large genera such as *Chironomus*. Therefore, while the scanning electron microscope would be a valuable tool in research comparing kinds and degrees of deformities, it is probably not practical for routine monitoring of environmental quality.

The background incidence of deformity found in this study, about 5–7%, is sharply higher than that reported in other regions. In rivers draining a rural area of Québec, Bird (1994) reported mean deformity frequencies of 0.8 – 2.5% at agricultural sites or those receiving municipal sewage effluent, and zero at clean reference sites. Swansburg et al. (2002) report mean deformity frequencies across many genera of 1.4% in streams receiving metals-mine drainage and 0.8% at reference sites. Other authors have reported incidences of mentum deformities at unpolluted sites of < 1% (Wiederholm, 1984; Warwick et al., 1987). Incidences of deformity reported here (3.8 to 14.9%) were always greater than these figures, even at seemingly unimpaired sites, and were relatively insensitive to the number of animals examined (43 to 456 at each site). This study did not include Tanypodinae in the calculation of deformity frequencies. Doing so would slightly reduce the calculated incidence (2.3 to 14.7%), assuming there are no deformed Tanypodinae, but would still leave frequencies noticeably higher than in comparable studies elsewhere.

None of the sites used in this study could be considered truly pristine; all lie within a highly managed landscape of second-growth forest and farmland. Pasture and hay are the most common crops, so contamination by agricultural chemicals is unlikely. There are few obvious sources of contamination in the reach of Ogdens Brook from which chironomids were collected, which is surrounded by unbroken forest. The headwaters of this stream, however, some 10 km upstream, rise in a high valley where land clearing for houses, pasture, and short-rotation forestry is common. Chironomids in Ogdens Brook, as in the other streams in this region, may be responding to broad-scale, mild impairment from historical and current land use.

There are clear differences among genera in their incidence of deformity at sites which we perceive to be uncontaminated: from near 15% in *Dicrotendipes, Endochironomus* and *Polypedilum*, to 2.4% in *Orthocladius* and *Tvetenia*, to zero in *Asheum* and one species of *Chironomus*. Therefore, differences in species composition among sites confound attempts to detect differences in deformation frequency arising from contamination. Taxon-by-taxon comparisons using a paired *t*-test or equivalent are possible only for those species present at both locations. This difficulty is compounded by the fact that characteristics of the mentum are of taxonomic value and a few larvae with severe deformation of the mentum cannot be confidently assigned to genus. However, most sites support several genera of chironomid, especially in lotic ecosystems; the average incidence of deformation across taxa is probably still a useful measure.

Combining data on mouthpart deformities from several species may be expedient where population densities are low. To reliably detect differences within single taxa at low deformation frequencies may require 100 to 200 animals per site (Bird, 1994; Swansburg et al., 2002), which would not be practical in the unproductive streams of this region. Low population densities do limit the accuracy of some of the estimates of deformity frequencies reported here. However, low numbers of animals at some sites are probably themselves an indication of poor habitat quality or chronic mortality. Finding one or two deformed animals in a sample of only a few is statistically unlikely unless the incidence of deformity in the population is high.

Average frequencies of deformity across many chironomid species were sufficient to confirm a rather small difference in deformation frequency below the Antigonish sewage effluent outfall. There was good congruence at that site among different measures of influence: chironomid community structure, incidence of deformities, and mass loss from decomposing leaf litter. All these attributes suggest a mild, negative effect of the effluent on benthic organisms, from insects to microbes. Given that the effluent is largely free of industrial waste, contains high concentrations of N and P (which would be expected to stimulate decomposition) and is free of acute toxicity in laboratory tests, the component responsible for the detrimental effect is a mystery. Of more interest here is the finding that mentum deformities in a mixed community of chironomids proved a sensitive indicator, complementary with other methods, with which to detect and quantify that impairment.

The population density of chironomids below the effluent outfall was much lower than above. This difference, itself probably an effect of the effluent, was offset through the use of litter bags to create an attractive habitat and stimulate colonization. The litter bags simultaneously provide a third, complementary indicator of effluent effects, as suggested by Gessner & Chauvet (2002). Hence, by combining mass loss, community structure and mentum deformities, a "weight-of-evidence" argument may be constructed that is more robust than conclusions based on one indicator alone (cf. Canfield et al., 1996).

Conclusions

Contrary to expectations based on previous work, the incidence of deformities in chironomid menta in northern Nova Scotia rivers appears to be rather high, on the order of 5–7% across all genera. These high deformity frequencies suggest a general, mild stress affecting water bodies in this area. Deformity frequencies varied widely among genera of chironomids, and the distribution of genera was scattered, with most occurring at only a few sites. In diverse, unproductive rivers such as these, comparing sites based upon incidence of deformity in the entire chironomid community may be the only feasible approach. There is a real advantage to combining incidence of deformity with changes in community structure, or functional measures such as decomposition rate, to assess environmental quality. Conventional microscopy appears to be as effective as the scanning electron microscope for enumerating deformities in large numbers of chironomid larvae.

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